ISSN: 0974 – 3987
IJBST (2017), 10(5):30-42

Biomass and genetic variability among *Telfaira occidentalis, Celosia argentea and Talinum triangulare* harvested from Okitipupa, Nigeria studied by random amplified polymorphic DNA (RAPD)

B.K. Bello, F. Adetuyi, O. Oluwaseun, O. Anthonia and S. Adegoke
Department of chemical sciences, Faculty of science, Ondo State University of Science and Technology, Okitipupa, Nigeria
tunlapa2k3@yahoo.com, foluadetuy@yahoo.co.uk, ossaitonia20@yahoo.com, oluwaseun222@yahoo.com, gokeadeosus@yahoo.com

Abstract:
Random Amplified Polymorphic DNA (RAPD) technique can be considered to be an important tool in phylogenetic studies, species identification, and assessment of genetic variability. In the present study, a "RAPD" technique was utilized to detect genetic variability among *Telfairia occidentalis*, *Celosia argentea* and *Talinum triangulare* harvested from selected locations in Okitipupa metropolis. A total sum of 47 bands was obtained in all the samples of *Telfaira occidentalis* amplified using the 5 RAPD arbitrary primers, for *Celosia argentea* a sum total of 26 bands was obtained in all the samples amplified, while a total sum of 61 bands was obtained in all the samples of *Talinum triangulare* amplified. The bands obtained ranged in size from 100 - 1200 bp. The extinction ratio (260/280 nm) of the isolated DNA was in the range from 1.5 to 2.5, indicating that the DNA was pure enough for RAPD analysis. Investigation of the plant biomass displayed evidence of reduced biomass accumulation. The results of this study suggested that RAPD technique can be used to detect polymorphism and genetic biodiversity in plant species.

Key words: *Telfaira occidentalis,* polymorphism, RAPD, *Celosia argentea* and *Talinum triangulare*

INTRODUCTION

Vegetables such as *Telfaira occidentalis, Celosia argentea* and *Talinum triangulare* are important part of healthy consumption and offer a source of many nutrients, including potassium which may assist in maintaining healthy blood pressure, dietary fiber which may assist in reducing blood cholesterol levels and may reduce risk of heart disease and folic acid which helps the body to produce good red blood cells. *Telfairia occidentalis* is a crop of commercial importance cultivated in West Africa (Nigeria, Ghana and Sierra Leone), being the major producers [1]. It is commonly called Fluted pumpkin and belongs to the family Cucurbitaceae, genus: telfairia and class: magnoliopsida-dicotyledons. It is a tropical crop which constitutes an important component of the diet of many people in West Africa countries [1]. The leaves are good source of vitamin, protein and minerals, which protect and nourishes the body, the leaves due to the richness in iron, it is used to cure anaemia [2], because of the good taste of the young shoot and leaves of the plants they are also used to prepare soup [3], the seeds provide an appreciable cash income to a small scale farmer, In Nigeria, the herbal preparation of the plant has been employed in the treatment of sudden attack of convulsion, malaria and anaemia [4]. Considering the oil content, the fluted gourd is high in oil (30%) [5]. Shoots of the plant contain high amount of potassium and iron, while seeds are composed of 27% crude proteins and 53% fats [6]. The leaves are rich in antioxidants and hepatoprotective and antimicrobial properties [7]. Waterleaf (*Talinum triangulare*) is a vegetable crop that belongs to the Portulacea family, Genus:Talinum Adans and class: magnoliopsida-dicotyledons it originated from tropical Africa and is popularly cultivated in West Africa, Asia, and South America [8]. In Nigeria, it is used in the preparations of 'Afang', Edikaiko and 'Gbure' soups which are indigenous to the Efiks, Ibibios and Yorubas, respectively. Water leaf is a good source of β-carotene, vitamins, pectin, proteins and minerals. Considering the nutritional content, waterleaf has been proven to be rich in protein ash, and crude fiber. It also has some medicinal values in humans and used as forage for rabbit feeding [9;10]. Waterleaf cultivation provides a good source of income to small-scale farming households [11]. It has also been observed to possess valuable health potentials such as purgative, laxative, diarrhea treatment, gastro-intestinal diseases [12;13] as well as in the treatment of diseases such as obesity and stroke [14].

Celosia argentea usually known as pumped cockscomb is an edible species which belongs to the genus *Celosia* of the Amaranthaceae family, and class: dicotyledoneae. It is popularly grown in some parts of West Africa. The leaves and flowers are used in Africa and Southeast Asia due to the edible

ISSN: 0974 – 3987
IJBST (2017), 10(5):30-42

nature [15]. The leaves contain high levels of calcium, phosphorus and iron. *Celosia argentea* is a good source of proteins, calories, vitamins and minerals that enrich the diet of West Africa people. Its liquid extract is used as a body wash for convalescents In Kenya [16]. The seeds are used as medicine for the treatment of diarrhea, dysentery and muscle troubles in Ethiopia and Democratic Republic of Congo [17;18]. The leaves mixed with honey are applied to inflated areas and the seeds are used for the treatment of diabetes mellitus in lndia [19].

Recently, the advent of molecular marker technology has provided new tools for detection of genetic alteration in response to environmental pollutants by looking directly at the level of DNA sequence and structure. Random Amplified polymorphic DNA technique (RAPD) constitutes a useful technique for the study of genetic polymorphism of DNA. RAPD involves the amplification of random segments of genomic DNA by Polymerase Chain Reaction (PCR), using short single primers of arbitrary sequence [20]. It is extremely effective for DNA analysis in complicated genomes as it is relatively cheap and yields information on a large number of loci without having to obtain sequence data for primer design [21]. Rapid Amplified Polymorphic DNA (RAPD) profiles are realized by PCR with single short primers of arbitrary nucleotide sequence. The resulting DNA profiles may be as a result of band shifts, missing bands or the appearance of new bands. Genetic variability can be evaluated by analyzing these bands. It can also be used to identify various types of DNA damage and mutations (e.g., point mutation, rearrangements, insertions or deletions of DNA and ploidy changes). They may hypothetically form the source of novel biomarker assays for the detection of DNA damage and mutations in cells of bacteria, plants and animals [22;23]. Biomass is the organic materials produced by plants such as leaves, roots and seeds. Environmental pollution has been shown to have unfavourable effects on plant growth and these may range from morphological abnormality, reduction in the biomass to stomatal aberrations [24].

In recent years, attention has been given to investigating the effect of genotoxic agents or pollutants on the genetic content and biomass of living organism. The difficulties encounter through direct analysis of pollutants effect on the genome of organisms and the interpretation of such measurements in terms of bioavailability have stimulated strong interest in analyzing Genetic variability among *Telfaira occidentalis, Celosia argentea and Talinum triangulare* from selected location in Okitipupa Metropolis Southwestern Nigeria owing to the increase in consumption of the selected vegetables in the metropolis and the pollution ravaging this metropoly which ranged from anthropogenic and sewage discharge in the ebute region to heavy metals pollution in the Oluwa glass industry region and palm oil mill effluent from Okitipupa oil mill industry environment which have the potential to disrupt genetic content and modify the natural growth of plants and alter significantly the plant content and diversity in the soil.

MATERIALS AND METHOD

Plant sample preparation

Telfaira occidentalis, Celosia argentea and Talinum triangulare were collected from four different selected locations (ebute-river, Okitipupa oil palm, oluwa glass environment and Ondo State University of Science and Technology farm) in Okitipupa Metropolis south western Nigeria. After harvesting, plants were washed, blotted to remove excess water and transported in liquid nitrogen to laboratory for DNA extraction.

DNA extraction

Total genomic DNA was extracted using the modified minipreparation protocol described by [25]. The PCR cocktail contains: 10× PCR buffer 1.0; 25 mM Mgcl2 1.0; 5 pMol primer 0.5; DMSO 1.0; 2.5 Mm DNTPs 0.8; Taq 1u/l 0.3; 10 ng/µl 2.0; H_2O 2.9. The cycling parameters for RAPD PCR were 94^0C, 3 min; 94^0,20 sec; 38^0C, 20 sec; 72^0C, 1 min and 72^0C, 7 min.

Sample preparation and gel electrophoresis

The mixture contains 2 µl dye and 4 µl of the PCR product which was loaded on the well of 1.5% Agarose gel. The sample was run for 1hour 30 min at 80 volt, 300 mA 60 Watt in gel electrophoresis machine. The plate was then dismantle and the gel was placed in Ethidium bromide solution for 5min after which it was visualized under UV trans-illuminator.

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Table 1: Operon primer sequences used for the RAPD-PCR analysis

Sequence number	Primer ID	Primer sequence
1	OPT-01	5‘ GGGCCACTCA 3‘
2	OPH-05	5‘ AGTCGTCCCC 3‘
3	OPT-06	5‘ CAAGGGCAGA 3‘
4	OPT-07	5‘ GGCAGGCTGT 3‘
5	OPT-20	5‘ GACCAATGCC 3‘

Table 1: Operon primer sequences used for the RAPD-PCR analysis. OPT denotes primers from Operon technologies, Kit T, while OPH is from Kit H.

Operon primer sequences used for the RAPD-PCR analysis and quantitative analysis of DNA

Table 1 depict the operon primer sequences used for the RAPD-PCR analysis. OPT denotes primers from Operon technologies, Kit T, while OPH is from Kit H. The purity and quantity of the isolated DNA was determined using a Nanodrop spectrophotometer. The extinction ratio (260/280 nm) was found between 1.5 to 2.0 an indication that the DNA was pure enough for RAPD analysis.

Biomass accumulation

Seeds were grown in the selected polluted environment under normal conditions except those grown in the University environment which was considered to be free from pollution. Plants were harvested for biomass measurements after 4 weeks of germination. The fresh weight of each individual plant leave, shoot and root was measured immediately after harvesting. For fresh weight biomass, the fresh weight of the leaf, shoot and root were measured immediately after harvesting, and the dry weight was recorded in (g) after drying in an oven to a constant weight at 70^0 C for 48 h.

Statistical analysis

All samples were analyzed in triplicates. The average and standard deviation of the mean as the error bar was calculated using Graphpad prism 5. Statistical analysis was made using statistical analysis software. (SAS 9.2). Data were analysed using analysis of variance (ANOVA).

RESULTS

RAPD agarose gel electrophoresis profile of the harvested *Telfairia occidentalis*, *Celosia argentea and Talinum triangulare* from selected locations in Okitipupa

The RAPD technique was used to analyze the extent of genetic diversity in *Telfairia occidentalis*, *Celosia argentea and Talinum triangulare* harvested from selected location in Okitipupa metropolis. Primers OPT–01, OPH–05, OPT–06, OPT–07, OPT–20 were used for amplification. Maximum number of bands were observed in *Telfaira occidentalis* obtained from ebute river (A4) *w*ith primer OPT–01 (4bands) followed by OPT–06 (3bands), OPT–20 (2bands) and OPT – 07 (1band), while there was no amplification in OPH–05. A sum total of 10 bands were amplified with respect to all the 5 primers. For *Telfaira occidentalis* obtained from Oluwa glass(A6) Maximum number of bands were observed *w*ith primer OPH – 05 (4 bands) and OPT – 20 (4 bands) followed by OPT – 06, (3 bands) and OPT – 01, (3 bands) and OPT–7 with the lowest bands (2 bands), A sum total of 16 bands were amplified with respect to all the 5 primers. Maximum number of bands was observed in *Telfaira occidentalis* obtained from University farm (A9) using primer OPT – 01 (4 bands) followed by OPH – 05 (3 bands), OPT – 06 and OPT – 20 (2 bands),with OPT – 7 producing the lowest band (1 band). A sum total of 12 bands were amplified with respect to all the 5 primers. The amplified product obtained for *Telfaira occidentalis* obtained from oil palm (A10) produced a sum total of 9 bands. Primer OPT – 01 produced the maximum number of bands (4bands) followed by OPT – 06 (2 bands) with OPH – 05, OPT – 07, OPT – 20 producing 1 band each respectively. Table 2

Maximum number of bands were observed in *Celosia argentea* obtained from University farm (A3) *w*ith primer OPT – 01 (3 bands) followed by OPH – 05 and OPT – 20 (1 band) whereas there was no amplification in OPT – 06 and OPT- 07. A sum total

ISSN: 0974 – 3987
IJBST (2017), 10(5):30-42

of 5 bands were amplified with respect to all the 5 primers. For *Celosia argentea* obtained from Oluwa glass company (A7), maximum number of bands were observed *w*ith primer OPT – 01, (6 bands), followed by OPT – 20 (4 bands) with OPH – 05, OPT – 06, and OPT – 7 producing 1 band each respectively. A sum total of 13 bands were amplified with respect to all the 5 primers. Maximum number of bands was observed in *Celosia argentea* obtained from Ebute river side (A8) using primer OPT – 01 (4 bands) followed by OPT-20 (2 bands), while no amplification product was recorded for primers OPH-05, OPT-06 and OPT-07. A sum total of 6 bands were amplified with respect to all the 5 primers. The amplified product recorded for *Celosia argentea* obtained from Oil Palm Mill (A12) produced a sum total of 2 bands. Primer OPT – 01 produced the only 2 bands whereas no amplification product was observed using OPT – 06, OPH – 05, OPT – 07 and OPT – 20. Table 2.

Maximum number of bands were observed in *Talinum triangulare* obtained from ebute river (A1) *w*ith primer OPT – 01 (4bands) followed by OPT – 20 (3bands), OPT – 6 and OPT-07 produced 2 bands each respectively, whereas there was no amplification product using OPH – 05. A sum total of 11 bands were amplified with respect to all the 5 primers. For *Talinum triangulare* obtained from University farm (A2), maximum number of bands were observed *w*ith primer OPT – 1 (6 bands) followed by OPT – 06, OPH – 05 and OPT-20 (4 bands) each. While OPT – 7 produced the lowest bands (2 bands), a sum total of 20 bands were amplified with respect to all the 5 primers. Primer OPT – 06 and OPT – 20 (5 bands each respectively) produced maximum number of bands in *Talinum triangulare* obtained from Oluwa glass (A5), primer OPH – 05 and OPT – 1 (3 bands each) while OPT – 07 produced the lowest band (2 bands). A sum total of 18 bands were amplified with respect to all the 5 primers. The amplified product obtained for *Talinum triangulare* harvested from oil palm (A11) produced a sum total of 12 bands. Primer OPT – 06, OPT – 01 and OPT – 07 produced the maximum number of bands (3 bands each) followed by OPT – 20 (2 bands) while OPH – 05 produced only 1 band. Table 2. The bands obtained ranged in size from 100 - 1200 bp. Figure 2-6.

Table 2. Total number of bands produced by the RAPD amplification of *Telfaira occidentalis, Celosia argentea* and *Talinum triangulare*

S/N	SAMPLES	OPERON CODE					
		OPT-01	OPH-05	OPT-06	OPT-07	OPT-20	TOTAL
1	(A4)	4	0	3	1	2	10
2	(A6)	3	4	3	2	4	16
3	(A9)	4	3	2	1	2	12
4	(A10)	4	1	2	1	1	9
							47
5	(A3)	3	1	0	0	1	5
6	(A7)	6	1	1	1	4	13
7	(A8)	4	0	0	0	2	6
8	(A12)	2	0	0	0	0	2
							26
9	(A1)	4	0	2	2	3	11

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10	(A2)	6	4		4	2	4	20
11	(A5)	3	3		5	2	5	18
12	(A11)	3	1		3	3	2	12 61

Table 5. Total number of bands produced by the RAPD amplification of *Telfaira occidentalis*, *Celosia argentea* and *Talinum triangulare* from selected location in Okitipupa metropolis.
Key:
Telfaira occidentalis from ebute river (A4), *Telfaira occidentalis* from Oluwa glass(A6)
Telfaira occidentalis from University farm (A9), *Telfaira occidentalis* from oil palm mill(A10)
Celosia argentea from University farm (A3), *Celosia argentea* from Oluwa glass (A7)
Celosia argentea from ebute river (A8), *Celosia argentea* from Oil palm mill (A12)
Talinum triangulare from ebute river (A1), *Talinum triangulare* from university farm(A2)
Talinum triangulare from oluwa glass (A5), *Talinum triangulare* from oil palm mill (A11)

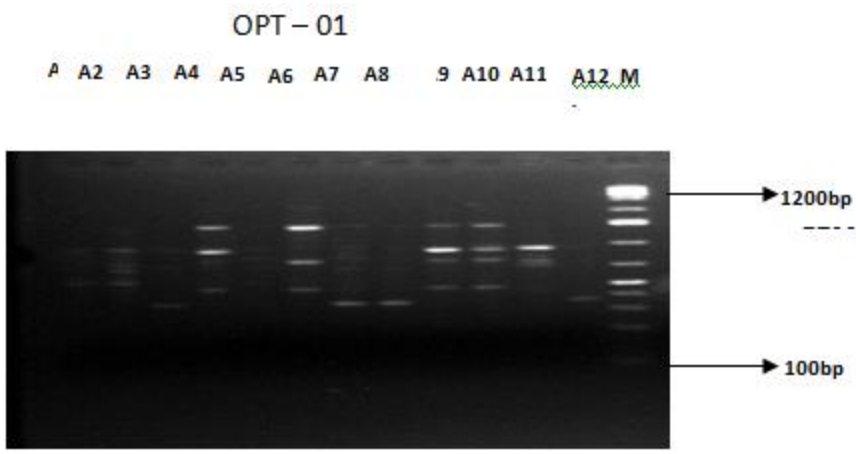

Figure 2. RAPD agarose gel electrophoresis profiles of the *Telfaira occidentalis, Celosia argentea and Talinum triangulare* harvested from selected location in Okitipupa metropolis using primer OPT-01. lane M: 1 kb DNA ladder; lane A1: *Talinum triangulare* from ebute river; lane A2: *Talinum triangulare* from University farm; lane A5: *Talinum triangulare* from Oluwa glass; lane A11: *Talinum triangulare* from oil palm mill. lane A4: *Telfaira occidentalis sp*. from ebute river; lane A6: *Telfaira occidentalis sp*. from Oluwa glass; lane A9:*Telfaira occidentalis sp*. from University farm; lane A10: *Telfaira occidentalis sp*. from oil palm mill. lane A3: *Celosia argentea* from University farm; lane A7: *Celosia argentea* from Oluwa glass company; lane A8: *Celosia argentea* from Ebute river side; lane A12: *Celosia argentea* from Oil palm mill.

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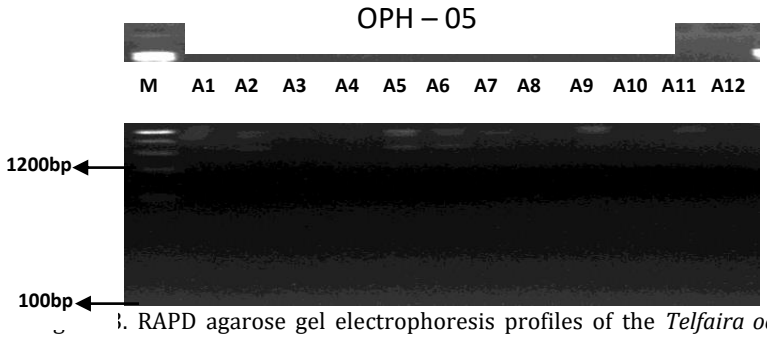

Figure 3. RAPD agarose gel electrophoresis profiles of the *Telfaira occidentalis, Celosia argentea and Talinum triangulare* harvested from selected location in Okitipupa metropolis using primer OPH-05. lane M: 1 kb DNA ladder; lane A4: *Telfaira occidentalis* from ebute river; lane A6: *Telfaira occidentalis* from Oluwa glass; lane A9:*Telfaira occidentalis* From University farm; lane A10: *Telfaira occidentalis* from oil palm Mill. lane A3: *Celosia argentea* from University farm; lane A7: *Celosia argentea* from Oluwa glass company; lane A8: *Celosia argentea* from Ebute river side; lane A12: *Celosia argentea* from Oil Palm Mill. lane A1: *Talinum triangulare* from ebute river; lane A2: *Talinum triangulare* from University farm; lane A5: *Talinum triangulare* From Oluwa glass; lane A11: *Talinum triangulare* from oil palm Mill.

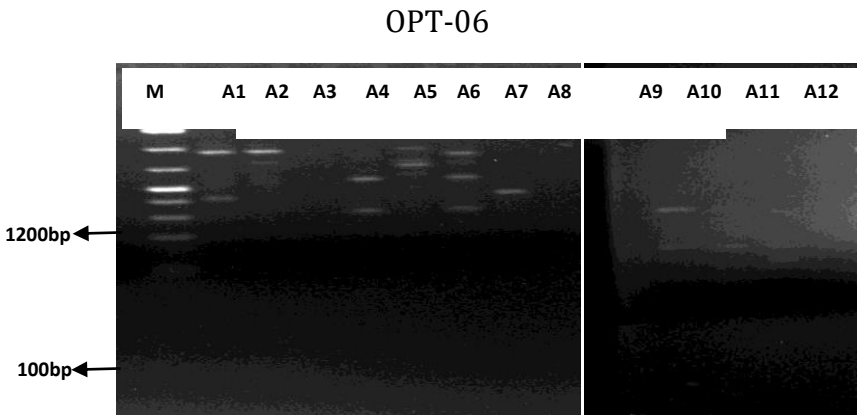

Figure 4. RAPD agarose gel electrophoresis profiles of the *Telfaira occidentalis, Celosia argentea and Talinum triangulare* harvested from different location in Okitipupa metropolis using primer OPT-06. lane M: 1 kb DNA ladder; lane A4: *Telfaira occidentalis sp*. from ebute river; lane A6: *Telfaira occidentalis sp*. from Oluwa glass; lane A9:*Telfaira occidentalis sp*. from University farm; lane A10: *Telfaira occidentalis sp*. from oil palm. lane A3: *Celosia argentea* from University farm; lane A7: *Celosia argentea* from Oluwa glass company; lane A8: *Celosia argentea* from Ebute river side; lane A12: *Celosia argentea* from Oil Palm Mill. lane A1: *Talinum triangulare* from ebute river; lane A2: *Talinum triangulare* from University farm; lane A5: *Talinum triangulare* From Oluwa glass; lane A11: *Talinum triangulare* from oil palm.

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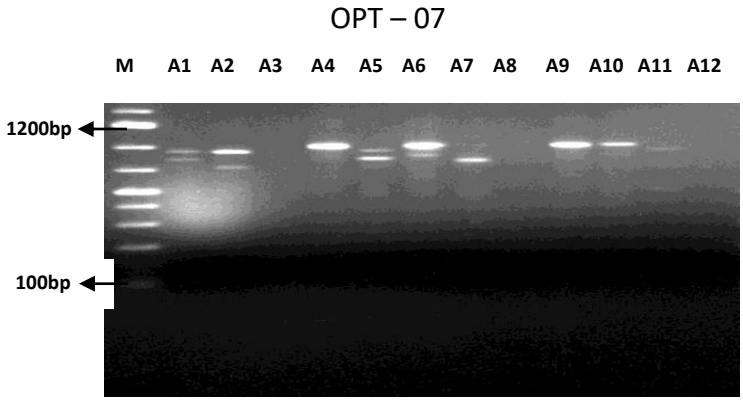

Figure 5. RAPD agarose gel electrophoresis profiles of the *Telfaira occidentalis, Celosia argentea and Talinum triangulare* harvested from selected location in Okitipupa metropolis using primer OPT-07. lane M: 1 kb DNA ladder; lane A4: *Telfaira occidentalis* from ebute river; lane A6: *Telfaira occidentalis* from Oluwa glass; lane A9:*Telfaira occidentalis* From University farm; lane A10: *Telfaira occidentalis* from oil palm mill. lane A3: *Celosia argentea* from University farm; lane A7: *Celosia argentea* from Oluwa glass company; lane A8: *Celosia argentea* from Ebute river side; lane A12: *Celosia argentea* from Oil Palm Mill. lane A1: *Talinum triangulare* from ebute river; lane A2: *Talinum triangulare* from University farm; lane A5: *Talinum triangulare* From Oluwa glass; lane A11: *Talinum triangulare* from oil palm mill.

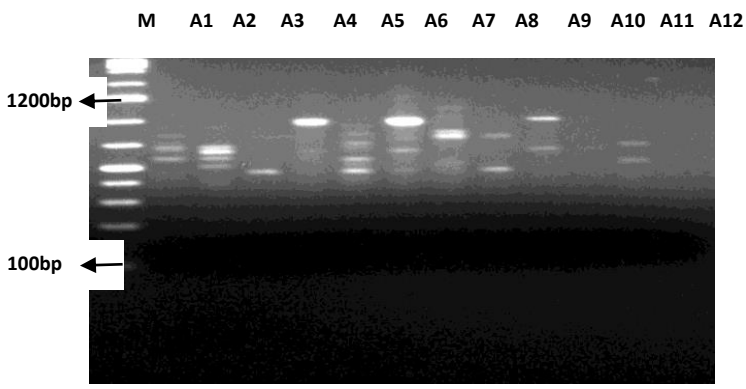

Figure 6. RAPD agarose gel electrophoresis profiles of the *Telfaira occidentalis, Celosia argentea and Talinum triangulare* harvested from selected location in Okitipupa metropolis using primer OPT-20. lane M: 1 kb DNA ladder; lane A4: *Telfaira occidentalis* from ebute river; lane A6: *Telfaira occidentalis* from Oluwa glass; lane A9: *Telfaira occidentalis* From University farm; lane A10: *Telfaira occidentalis* from oil palm. lane A3: *Celosia argentea* from University farm; lane A7: *Celosia argentea* from Oluwa glass company; lane A8: *Celosia argentea* from Ebute river side; lane A12: *Celosia argentea* from Oil Palm Mill. lane A1: *Talinum triangulare* from ebute river; lane A2: *Talinum triangulare* from University farm; lane A5: *Talinum triangulare* From Oluwa glass; lane A11: *Talinum triangulare* from oil palm mill

Biomass accumulation of *Talinum triangulare* harvested from selected location in Okitipupa metropolis

The weight of the fresh and dry sample of *Talinum triangulare* harvested from selected location in Okitipupa metropolis varied statistically ($p \leq 0.05$). The fresh weight of the leaves of *Talinum triangulare* obtained from ebute-river, oluwa glass, oil palm and OSUSTECH farm were recorded as 2.71g, 2.83g, 3.11g and 3.22g respectively, with that of Ebute-river recording the lowest weight (2.7g), while that of OSUSTECH farm recorded the highest weight (3.2g). The fresh weight of the stems of *Talinum triangulare* obtained from this same locations, were recorded as 5.00g, 5.11g, 5.49g and 5.61g respectively for ebute-river, oluwa glass, oil

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palm and OSUSTECH farm, with the highest value obtained from OSUSTECH farm (5.61g) followed by oil palm (5.5g). The least value was obtained from Ebute-river (5.00g). 3.89g, 4.00g, 4.11g, 4.32g were recorded for the fresh weight of root from Ebute-river, oluwa glass, oil palm, and OSUSTEC farm locations respectively. The fresh weight of root from OSUSTECH farm gave the highest value (4.32g), followed by oil palm (4.11), oluwa glass (4.00) and Ebute-river (3.89g). The dry weight of the leaves, stems and root of the *Talinum triangulare* obtained from ebute-river, oil palm, oluwa glass and OSUSTECH farm were also recorded. The dry weight of the leaves obtained from these four (4) locations were 1.11g, 1.52g, 1.63g and 2.11g for ebute-river, oluwa glass, oil palm and OSUSTECH farm respectively, while that from OSUSTECH farm recorded the highest value (2.11g), that of ebute-river recorded the lowest (1.11g). The dry weight of the stems from this same locations were 1.33g, 2.0g, 2.33g and 2.55g respectively for ebute-river, oluwa glass, oil palm, and OSUSTECH farm, with the weight obtained from ebute-river (1.33g) being the lowest and the weight obtained from OSUSTECH farm (2.55g) being the highest. Dry weight of root sample collected from the four location gave the following values: 1.23g, 1.96g, 2.20 and 2.41g 0.9g for Ebute river, Oluwa River, oil palm, and OSUSTECH farm respectively. Fig. 7

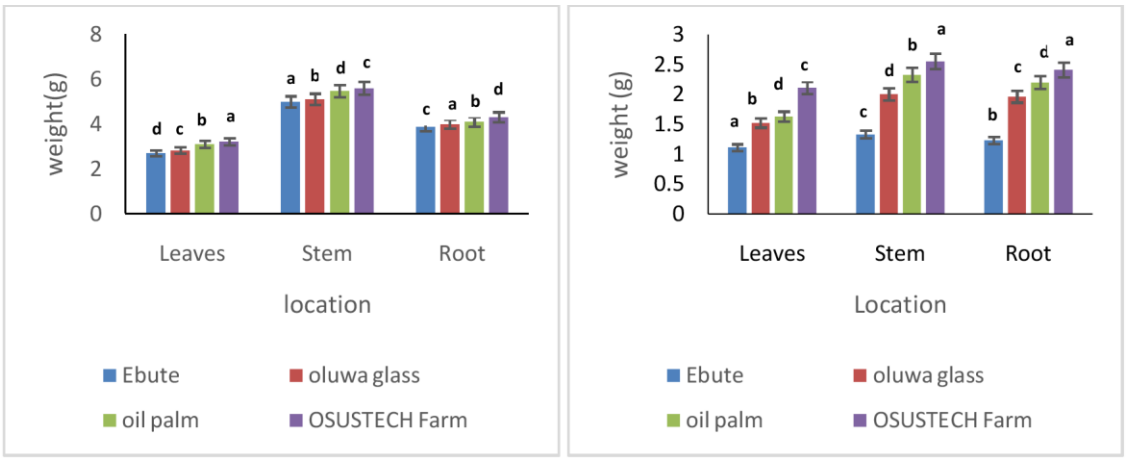

Figure 7: Biomass accumulation of *Talinum triangulare* sample obtained from selected location in Okitipupa metropolis. (A). Fresh weight biomass. The fresh weight of the stems, roots and leaves was immediately measured after harvesting. (B). dry weight biomass. The dry weight in the stems, roots and leaves was recorded after drying in an oven to a constant weight at 70^{0}C for 48 h. Values are means of three biological replicates. Means with different letters are significantly different ($p \leq 0.05$).

Biomass accumulation of *Celosia argentea* obtained from selected locations in okitipupa metropolis

The fresh weight of the leaves of *Celosia argentea* obtained from Ebute river side, Oil palm mill, Oluwa glass company and OSUSTECH farm were recorded as 1.60g, 1.80g, 1.70g and 1.90g respectively, with that of Ebute river side recording the lowest weight while that of OSUSTECH farm recorded the highest weight. The fresh weight of the stems of the vegetable obtained from this same locations, were recorded as 4.20g, 4.80g, 4.60g and 4.90g respectively for Ebute river side, Oil palm mill, Oluwa glass company and OSUSTECH farm. The fresh weight of the roots were recorded as 3.90g, 3.10g, 2.80g, 3.20g respectively for Ebute river side, Oil palm mill, Oluwa glass company, and OSUSTECH farm. Likewise, the weight of the dried leaves stems and roots of the sample obtained from Ebute river side, Oil palm mill, Oluwa Glass Company and OSUSTECH farm were recorded. The dry weight of the leaves obtained from these four (4) locations were 0.40g, 0.50g, 0.40g and 0.60g respectively for Ebute river side, Oil palm mill, Oluwa glass company and OSUSTECH farm, while that of OSUSTECH farm recorded the highest value (0.60g), that of Ebute river side and Oluwa glass company recorded the lowest (0.40g each). The dry weight of the stems were 0.80g, 1.00g, 0.90g and 1.10g respectively for Ebute river side, Oil palm mill, Oluwa glass company and OSUSTECH farm, with the weight obtained from Ebute river side being the

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lowest and the weight obtained from OSUSTECH farm being the highest. The dry weight of the roots were recorded as 1.00g, 0.80g, 0.60g, 0.90g respectively for Ebute river side, Oil palm mill, Oluwa glass company, and OSUSTECH farm (Figure 8)

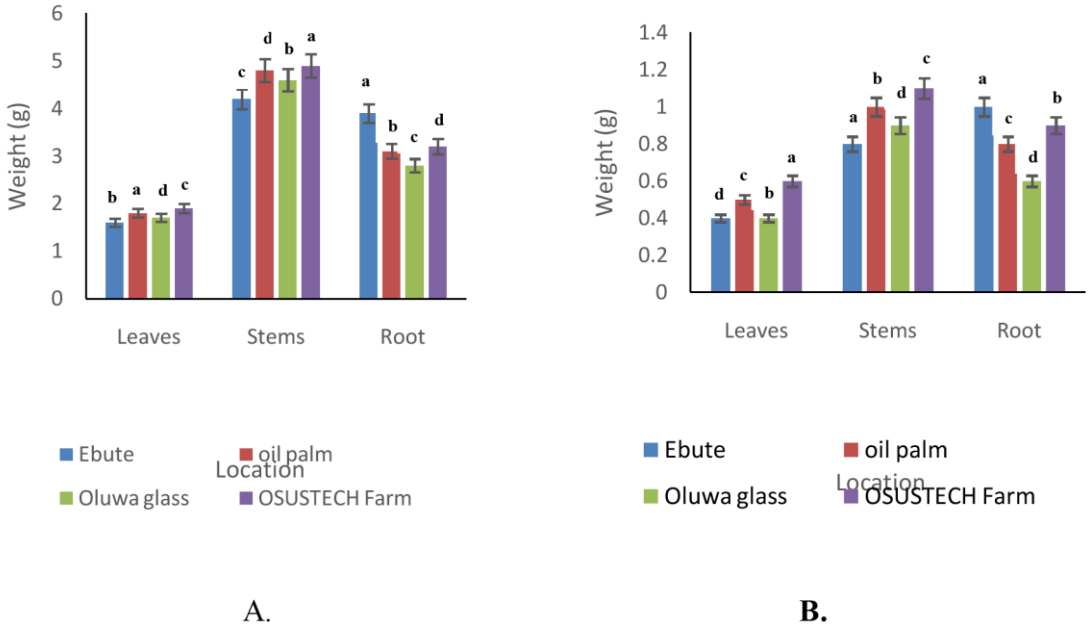

Figure 8: Biomass accumulation of *Celosia argentea* sample obtained from selected location in Okitipupa metropolis. (A). Fresh weight biomass. The fresh weight of the stems, roots and leaves was immediately measured after harvesting. (B). dry weight biomass. The dry weight in the stems, roots and leaves was recorded after drying in an oven to a constant weight at 70^{0}C for 48 h. Values are means of three biological replicates. Means with different letters are significantly different (p≤0.05).

Biomass Accumulation of *Telfairia occidentalis* harvested from selected location in Okitipupa metropolis.

The weight of the fresh and dry sample of *Telfairia occidentalis* harvested from different location in Okitipupa metropolis varied statistically (p≤0.05). The fresh weight of the leaves of obtained from ebute-river, oil palm, oluwa glass and OSUSTECH farm were recorded as 2.80g, 2.91g, 3.24g and 3.33g respectively, with that of ebute-river recording the lowest weight (2.80g), while that of OSUSTECH farm recorded the highest weight (3.33g). The fresh weight of the stems obtained from this same locations, were recorded as 5.13g, 5.24g, 5.70g and 5.81g respectively for ebute-river, oil palm, oluwa glass and OSUSTECH farm, with the highest value obtained from OSUSTECH fram (5.81g), while the lowest value was obtained from ebute-river (5.13g). The fresh weight of the roots were recorded as 4.00g, 3.20g, 3.50g, 3.80g respectively for Ebute river side, Oil palm mill, Oluwa glass company, and OSUSTECH farm. In the same vein, the dry weight of the leaves, stems and roots from the same location were recorded. The dry weight of the leaves of *Telfairia occidentalis* obtained from these locations were 0.64g, 0.81g, 0.92g and 1.00g respectively for ebute-river, oil palm, oluwa glass and OSUSTECH farm The dry weight of the stems were 1.10g, 1.20g, 1.30g and 1.40g respectively for ebute-river, oil palm, oluwa glass and OSUSTECH farm. The dried weight of the roots were recorded as 0.90g, 0.80g, 1.00g, 1.10g respectively for Ebute river side, Oil palm mill, Oluwa glass company, and OSUSTECH farm Figure. 9

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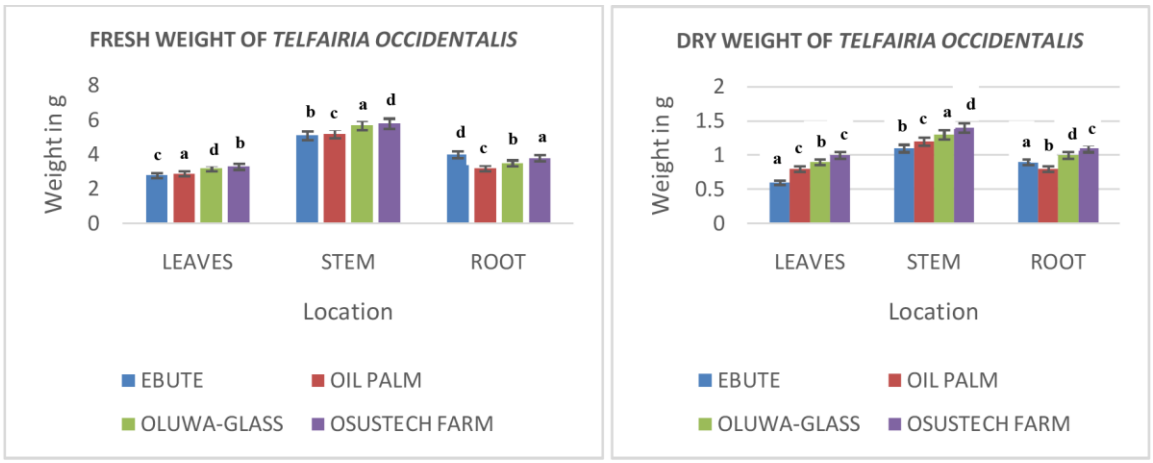

A B

Figure 9: Biomass accumulation of *Telfairia occidentalis* sample obtained from selected location in Okitipupa metropolis. (A). Fresh weight biomass. The fresh weight of the stems, roots and leaves was immediately measured after harvesting. (B). dry weight biomass. The dry weight in the stems, roots and leaves was recorded after drying in an oven to a constant weight at 70^{0}C for 48 h. Values are means of three biological replicates. Means with different letters are significantly different ($p \leq 0.05$).

DISCUSSION

The variation in genetic composition caused by genotoxic chemicals may be investigated using different biomarker assays both at biochemical and molecular level [22]. Random amplified polymorphic DNA (RAPD) profiles show variations in genomic DNA with the use of arbitrarily primer and visibly show promise in the detection of pollutant-influenced DNA alteration. Indeed, it is only a qualitative method through which nature and quantity of DNA can only be hypothesized. RAPD can be considered an important tool in species identification, assessment of genetic variability and relationships management of genetic biodiversity and resources, studies of phylogenetic relationships and in genome mapping [26]. In the present research, genetic variability among the *Telfaira occidentalis, Talinum triangulare* and *Celosia argentea* obtained from selected location in Okitipupa metropolis were analysed using random amplified polymorphic DNA technique. Primers OPT – 01, OPH – 05, OPT – 06, OPT – 07, OPT – 20 were used for amplification. DNAs of *Telfaira occidentalis, Talinum triangulare* and *Celosia argentea* obtained from selected location in Okitipupa metropolis were extracted using the modified minipreparation protocol described by [25] and tested by both Qualitative and Quantitative methods in order to obtain good DNA quality for RAPID analysis. Five random primers were used in this study these primers produced polymorphic amplification fragments that were highly reproducible and visible but revealed different intensity, band size, and numbers after the amplification products were visualized by separation on a 1.5% agarose gel stained with ethidium bromide and view under UV transilluminator. The variation in band intensity, band size, and numbers is an indication of genetic variability among *Telfaira occidentalis, Talinum triangulare* and *Celosia argentea* obtained from selected location in Okitipupa metropolis and this may be attributable to the effect of environmental pollution which have altered the genetic composition of the vegetable as the environment where the vegetable was harvested have been subjected to different kind of environmental pollution which could be anthropogenic pollution, exhaust gas from vehicles, sewage, heavy metal and palm oil effluent .

The RAPD polymorphisms observed among the *Telfaira occidentalis, Talinum triangulare* and *Celosia argentea* from selected location in Okitipupa metropolis could be attributable to a change in base which alters the binding site of the primer, or an insertion/deletion within the region amplified [20;27], polymorphisms are usually noted by the

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presence or absence of an amplification product from a single locus. The work in [28] reported DNA changes in RAPD profiles in roots and leaves of bean (*Phaseolus vulgaris* L.) exposed to toxic chemicals. The study of *Telfaira occidentalis, Talinum triangulare* and *Celosia argentea* harvested from selected location in Okitipupa metropolis confirms the suitability of RAPD as a reliable, simple and easy to handle technique in analyzing the genetic variability of different accessions of an important vegetable plant. Concurrently, it is also proved that the entries which were found to be similar in taxonomical classification were based on morphological characters. The study in [29] reported that polymorphisms became evident as the presence and/or absence of DNA fragments in heavy metals treated and control sample of *Phaseolus vulgaris L*. their results revealed a total of 295 RAPD fragments of 700-4000 bp in molecular size in the seedlings of untreated and treated samples, of which 163 fragments were polymorphic. In our findings, changes observed in the DNA profiles such as modifications in band intensity extra band and loss of bands may be due to the changes in oligonucleotide priming sites mainly due to genomic alteration, and not likely to point mutations or DNA mutilation in the primer binding sites or the presence of the product of DNA photochemical reaction which can hinder or reduced the polymerization of DNA in the PCR reaction. [30]. The work in [31] reported that A total of 467 RAPD fragments in RAPD profiles were detected by using six random primers and 224 of these fragments showed polymorphism after treatment of kidney-bean (Phaseolus vulgaris) with heavy metal. He further stated that there was a diverse distance between the band patterns of treated plants and the control samples when the cluster method was utilized. Our findings revealed appearance of new PCR products or appearance of bands which may be attributable to the existence of oligonucleotide priming sites which become approachable to oligonucleotide primers after structural alteration or due some changes in DNA sequence which might have occurred due to mutations resulting in new annealing pattern or considerable deletions bringing two pre-existing annealing sites closer or homologous recombination [21]. The result of our study corroborated the findings of [32] who reported that, results produced from nine primers indicate that the changes occurring in RAPD profiles of the root tips of barley seedlings following Cd treatment included alterations in band intensity as well as gain or loss of bands compared with the control seedlings. The work in [33] suggested that after proper optimisation, the RAPD is a reliable, sensitive and reproducible assay, has the potential to detect a wide range of DNA damage as well as mutations and therefore can be applied to genotoxicity findings. Occurrence of some extra band in this findings as can be observed in *Telfaira occidentalis* and *Talinum triangulare* harvested from ebuted using primer OPT-01, *Telfaira occidentalis*, *Talinum triangulare* and *Celosia argentea* harvested from Oluwa glass environment using primer OPT-01, OPH-05 and OPT-20, *Telfaira occidentalis* and *Talinum triangulare* harvested from University farm using primer OPT-01 and *Telfaira occidentalis sp* harvested from Oil palm environment using primer OPT-01 indicated that the effect can be involved in DNA repair and replication technique or it may also be the result of genomic template uncertainty related to the level of DNA damage and the conformity of DNA repair and replication [21]. Moreover, similar findings with respect to metal toxicity alteration in genetic pattern were also reported in *Arabidopsis thaliana* when subjected to Pb, Mn, Cd etc. [34] using the RAPD technique. Changes in DNA pattern were also observed under heavy metal stress in *Daphnia magna*, *Hordeum vulgare* and *Phaseolus vulgaris* [35;36;31]. Lead, copper, and cadmium affect genomic DNA of *Silene paradoxa*, kidney and barley plants [37;31;36]. Decrease in the biomass accumulation observed in the present findings may be attributable to chemical composition of soil, use of inorganic and organic fertilizer, anthropogenic factor and heavy metal pollution. The work in [38] reported that cadmium accumulation in maize plant resulted in decrease in the plant biomass. Copper accumulation has also been implicated in reduction of fruit weight in black blind weed. [39].

Our findings provided further evidence that RAPD technique offers reliable method for characterizing variations among species, within a species and among population of *Telfaira occidentalis, Talinum triangulare* and *Celosia argentea* harvested from selected location in Okitipupa metropolis.

CONCLUSION

The present study revealed that RAPD technique can be used for species identification and assessment of genetic variability among *Telfaira occidentalis, Talinum triangulare* and *Celosia argentea* harvested from selected location in Okitipupa metropolis. It is however suggested that after proper optimisation, RAPD is a reliable,

ISSN: 0974 – 3987
IJBST (2017), 10(5):30-42

sensitive and reproducible assay, has the possibility to detect a wide range of genetic variability, DNA damage (e.g. DNA adducts, DNA breakage) as well as mutations (point mutations and large rearrangements) and therefore can be applied to genotoxicity. Finally, to elucidate the effect of environmental contaminants on the genetic variability among *Telfaira occidentalis species*, a cogent strategy could be firstly to use the RAPD assay as a screening method and secondly to adopt more specific methods for instance measuring DNA adducts, gene mutations or cytogenetic.

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